currents supplied to the electric furnaces. This may have been in reality a subjective phenomenon similar to that now recorded.

No effect upon the senses of smell, taste, or hearing has yet been observed.

[*Added April* 14.—Since the above was written, it has been noted by several observers that a sensation of taste in the mouth is excited after exposing the head for two or three minutes to the action of the alternating magnetic field.]

On the Weight of Precipitate Obtainable in Precipitin Interactions.

By Dr. H. G. Chapman.

(Communicated by Dr. C. J. Martin, F.R.S. Received April 21,—Read May 5, 1910.)

(From the Physiological Laboratory of the University of Sydney.)

The nature of the interaction between the antiserum and homologous protein in a precipitin test has been the subject of several researches. It has been usual to mix a given fixed quantity of antiserum with increasing quantities of suitably diluted homologous protein estimated either directly or in terms of dilutions and to measure the volume of the precipitate formed. The experiments of Hamburger which have been analysed by Arrhenius* were conducted in this way. Welsh and Chapman† also examined mixtures of a fixed quantity of antiserum with increasing quantities of homologous protein by adding to the superfluid above the precipitate either antiserum or homologous protein. This mode of testing the superfluid after the completion of the precipitin reaction led to certain definite conclusions. In the first place the further addition of antiserum to the superfluid always led to the formation of a further precipitate. In the second place it was possible to neutralise completely the precipitin in an antiserum, so that the further addition of homologous protein led to no more formation of precipitate.

The precipitin reaction between antigen and anti-body is very suitable for quantitative study owing to the simple nature of the interaction and the ease with which the quantities may be measured. More accurate methods are, however, required for its study than those previously employed. The

* S. Arrhenius, 'Immuno-chemistry,' New York, 1907, p. 287 *et seq.*, and 'Ergebnisse d. Physiol.,' vol. 7, p. 543, 1908.

† 'Journ. of Hygiene,' vol. 6, p. 251, 1906.

present paper records the results of a gravimetric study of the reaction. The weights of the two interacting bodies and the weight of the final precipitate have been ascertained. The precipitin (anti-body present in the serum of the immunised animal) cannot be directly determined by weighing, as it forms only a small part of the dried antiserum. It will be assumed in the paper to be proportional to the volume of the antiserum. The homologous protein (either serum or egg-white) has been reckoned as milligrammes of dried serum or dried egg-white. The precipitate formed in the interaction has been weighed.

The most striking feature of the results obtained is the strict proportion of the weight of the precipitate to the amount of the antiserum, provided the quantity of the homologous protein exceed certain minimal amounts. If a quantity of dried horse serum such as 50 mg. be allowed to interact in suitable dilution with 1, 2, 3 and 4 c.c. antiserum for horse serum the weights of the precipitates will be in the ratio of 1, 2, 3 and 4. This fact stands in harmony with the evidence obtained by Welsh and Chapman* concerning the origin of the precipitate mainly from the antiserum. The quantity of precipitate represents the "precipitable content" of the antiserum and its weight is practically that of the precipitin present in the antiserum.

Methods.

Rabbits and cats were employed to produce the antisera. They received six to eight injections of serum or egg-white into the abdominal cavity. The quantity of material injected was determined by drying quantities of serum or egg-white to constant weight. Approximately 1 gramme was given at at each injection. The animals were killed by bleeding 12 to 16 days after the final injection. The serum was allowed to separate spontaneously. This serum was utilised at once for the experiments. The whole serum from any immunised animal was mixed together before use, as it was found that the weight of the precipitate from the antiserum first separated differed considerably from that of the antiserum separated later. All vessels, glass tubes, measures and pipettes were sterilised by steam immediately before use. The saline solution (0·75 per cent. NaCl) was sterilised by boiling thrice on successive days. The antiserum was measured by a pipette carefully graduated by weighing the amount of mercury delivered. The sera and egg-whites used as homologous proteins were similarly measured after suitable dilution. The solid content of the serum or egg-white was estimated by drying to constant weight a measured volume. In this way it was more easy to ensure the absence of bacterial contamination. The quantities of the

* 'Roy. Soc. Proc.,' B, vol. 78, p. 297, 1906.

reacting bodies were mixed with saline solution in large tubes for the centrifuge. The tubes were made up to a fixed volume with saline solution and allowed to stand 48 hours for the interaction to take place. The superfluid above the precipitate was removed with a pipette and the precipitate washed five times with saline solution. Each time the precipitate was mixed with 50 c.c. saline solution and the precipitate separated by spinning in the centrifuge. The precipitate was then washed five times in the same way with 50 c.c. distilled water. The precipitate was transferred to small glass tubes with thin walls, weighing about 4 grammes. These tubes could be spun in a small centrifuge and, in this way, the precipitate was washed with absolute alcohol and finally with ether free from water. The tubes with their contents were placed in an oven at 80° C. for several hours and thence were put in a desiccator. The tubes were kept a fixed time in the desiccator and weighed. The tubes of a series were weighed immediately after each other. Owing to the hygroscopic nature of the precipitates the error in the weights of the tubes was found by experiment to be 0·3 milligramme. The mean figure of the several weighings was taken in all cases.

Experimental Results.

Experiments were performed to ascertain the weight of precipitate obtained when a measured quantity of antiserum was allowed to interact with increasing weights of homologous protein. It has been shown by Welsh and Chapman* that no precipitin can be detected in the superfluid at the end of an interaction, provided that the amount of homologous protein exceeds a certain quantity. In the series to be described the quantity of protein was sufficient to neutralise or precipitate† the precipitin in the antiserum. The superfluids were considered free from precipitin, since they yielded no precipitate on the addition of 144 milligrammes dried egg-white. The results are recorded in Table I.

Table I.

No. of tube.	Weight of dried egg-white.	Volume of antiserum.	Volume of saline solution.	Weight of precipitate.	Weight of precipitate from 1 c.c. antiserum.
	milligrammes.	c.c.	c.c.	milligrammes.	milligrammes.
1	14·4	2	50	3·2	1·6
2	36·0	2	50	3·5	1·75
3	144·0	2	50	3·4	1·7
4	432·0	2	50	3·4	1·7

* 'Journ. of Hygiene,' vol. 6, p. 251, 1906.

† Compare Welsh and Chapman, 'Roy. Soc. Proc.,' B, vol. 78, p. 297, 1906.

Here the antiserum was formed by the injection of fowl's egg-white and the fresh antiserum allowed to interact with fresh egg-white. A portion of the egg-white was dried to determine the solid content of the solution. The precipitates are stated in terms of the amount yielded with 1 c.c. antiserum. The weight of precipitate remains practically constant, although the protein increases from 14 to 432 grammes. A somewhat similar series is recorded in Table II. In this series, 3 c.c. antiserum were allowed to interact with 70, 140, 280, and 560 milligrammes dried egg-white. After 24 hours the superfluids were removed, and to the superfluid of the tube No. 1 (1B in table), 70 milligrammes dried egg-white were added; to that of the tube No. 2 (2B in table), 140 milligrammes dried egg-white were added, and the remaining two tubes were tested in the usual way. The precipitates were treated in the manner above described, and weighed. No precipitates occurred in the secondary superfluids of tubes Nos. 3 and 4.

Table II.

No. of tube.	Weight of dried egg-white.	Amount of anti-serum.	Amount of saline solution.	Weight of precipitate.	Total weight of the precipitates.	Total weight of precipitate from 1 c.c. antiserum.
	milligrammes.	c.c.	c.c.	milligrammes.	milligrammes.	milligrammes.
1	70	3	50	2·8	3·5	1·16
1B	+ 70	—	—	0·7		
2	140	3	50	3·2	3·2	1·06
2B	+ 140	—	—	trace		
3	280	3	50	3·0	3·0	1·0
4	560	3	50	3·2	3·2	1·06

The superfluids from tubes 1B and 2B were tested for the presence of precipitin by the addition of more protein, but no precipitates were obtained. In this series the weight of precipitate obtained from 1 c.c. antiserum remains constant, despite the large increase in the quantity of protein. No stress can be laid on the amount of precipitate in tube No. 1B, since it is doubtful whether the interaction in tube No. 1 was complete in 24 hours.

As it appears that the precipitate from a given quantity of antiserum is constant, provided there be sufficient protein to neutralise the precipitin, a series of experiments in which the amount of antiserum was varied may be considered. With these experiments may be considered one in which a duplicate was carried out. The details of the experiments were varied to avoid errors. The results are recorded in Table III.

The quantity of protein was found to be sufficient to neutralise all the precipitin except in tubes Nos. 11, 12, and 13. The results show that the

Table III.

No. of tube.	Antiserum.	Amount of antiserum.	Weight of dried protein.	Amount of saline solution.	Weight of precipitate.	Weight of precipitate from 1 c.c. antiserum.
		c.c.	mgrms.	c.c.	mgrms.	mgrms.
1	Horse serum, 57...	2·5	100	50	3·7	1·5
2	" ...	2·5	100	50	3·5	1·4
3	Hen egg-white, 59	2·0	134	50	8·6	4·3
4	"	3·0	134	50	12·5	4·2
5	"	4·0	134	50	16·7	4·2
6	Horse serum, 53...	2·5	50	50	2·0	0·8
7	"	5·0	200	50	4·0	0·8
8	Horse serum, 56...	5·0	100	50	10·4	2·1
9	"	10·0	100	50	20·0	2·0
10	Hen egg-white, 64	1·0	28	50	1·4	1·4
11	"	2·0	28	50	2·2	1·35
11B	....	—	+140	—	0·5	
12	Hen egg-white, 64	3·0	28	50	3·2	1·4
12B	....	—	+ 56	—	1·0	
13	Hen egg-white, 64	4·0	28	50	3·6	1·5
13B	....	—	+ 56	—	2·4	

amount of precipitate yielded by each antiserum is a fixed quantity for each cubic centimetre of antiserum. It must be noted that the amount of saline solution used as a diluent is the same throughout the series. In tubes Nos. 1 and 2, 2·5 c.c. antiserum for horse serum interacted with 100 milligrammes dried horse serum and the duplicates agree well. In tubes Nos. 3, 4, and 5, 2, 3, and 4 c.c. fowl's egg-white antiserum interacted with 100 milligrammes dried egg-white, yielding 8·6, 12·5, and 16·7 milligrammes precipitate respectively. Calculating the amount for each cubic centimetre antiserum it is found to be about 4·2 milligrammes in each case. In tubes Nos. 6 and 7, 2·5 c.c. antiserum for horse serum reacted with 50 milligrammes horse serum, and 5 c.c. antiserum reacted with 200 milligrammes horse serum. The amount of precipitate in tube No. 7 was double that in tube No. 6. In tubes Nos. 8 and 9, 5 c.c. and 10 c.c. antiserum each interacted with 100 milligrammes dried egg-white and the weights of the precipitates agree sufficiently for each cubic centimetre. In tubes Nos. 10, 11, 12, and 13, 1, 2, 3, and 4 c.c. antiserum for fowl's egg-white each reacted with 28 milligrammes dried egg-white. After 48 hours the superfluids were removed and a quantity of dried egg-white dissolved in saline solution added to each superfluid. No further precipitate formed in tube No. 10, but precipitates formed in tubes Nos. 11B, 12B, and 13B. Here, again, there is fair agreement in the amount of precipitate finally obtained from each cubic centimetre of antiserum.

When the quantity of protein is not sufficient to neutralise all the precipitin in a given amount of antiserum the weight of precipitate is diminished. An experiment showing the relation of the precipitate to the amounts of the interacting bodies may be now described. A rabbit was immunised by the injection of 9·6 grammes dried egg-white in eight doses. The quantities employed and the results obtained are recorded in Table IV.

Table IV.

No. of tube.	Amount of antiserum.	Weight of protein.	Amount of saline solution.	Weight of precipitate.	Weight of precipitate from 1 c.c. antiserum.
	c.c.	milligrammes.	c.c.	milligrammes.	milligrammes.
1	3	1·44	50·0	1·0	0·33
2	3	3·6	50·0	1·5	0·5
3	3	7·2	50·0	2·0	0·66
4	3	14·4	50·0	2·7	0·9
5	3	28·8	50·0	4·2	1·4
6	3	144·0	50·0	6·5	2·2

It will be seen that the amount of antiserum was 3 c.c. in each tube. This quantity yields such small precipitates in the tubes Nos. 1 and 2 that great stress cannot be placed on these figures. The weight of the precipitates has steadily increased. The amount of antiserum obtained from a rabbit is not usually more than 20 c.c., so that extended series cannot be carried out with antisera from rabbits. It was not considered legitimate to employ mixed antisera. Other series gave similar results, but at present the data are too few to discuss these results at length to determine the type of the interaction.

The effect of the degree of dilution on the weight of precipitate may be now considered. Two series of experiments were carried out. In the first series the quantity of egg-white was constant and the amount of saline solution used to dilute the interacting masses was varied. In the second series the concentration of egg-white was maintained constant in the varying amounts of saline solution. The antisera employed were two fowls' egg-white antisera prepared from rabbits. The results are recorded in Table V.

The results of both series correspond, though the absolute amount of precipitate from each antiserum was different. With a quantity of saline solution of 25 c.c. there was a reduction in the weight of precipitate. All observers have noted this reduction, which has been usually ascribed to a solvent action of the concentrated serum. With a quantity of saline solution of 100 c.c. the weights of the precipitates were also slightly reduced. This reduction was probably due to incomplete reaction in 48 hours, since

the superfluids removed from tubes Nos. 3 and 6 yielded small precipitates on standing for another 48 hours.

Table V.

No. of tube.	Amount of antiserum.	Weight of egg-white.	Amount of saline solution.	Weight of precipitate.	Weight of precipitate from 1 c.c. antiserum.
	c.c.	milligrammes.	c.c.	milligrammes.	milligrammes.
1	5A	100	25	10·8	2·2
2	5A	100	50	16·2	3·2
3	5A	100	100	15·0	3·0
4	5B	100	25	10·6	2·1
5	5B	200	50	19·5	3·9
6	5B	400	100	17·7	3·6

The results recorded above are typical of those obtained by the examination of 23 different antisera. The amounts of precipitate obtained from the various antisera showed much variation. The quantity of homologous protein required to precipitate completely the precipitin showed similar variations. The gravimetric results confirm the volumetric results of Welsh and Chapman* as to (1) the amount of precipitable substance in an antiserum, and (2) the amount of homologous protein necessary to neutralise and precipitate this precipitable substance. The quantity of precipitate obtained from 1 c.c. antiserum on the complete precipitation of the precipitable substance of the antiserum is recorded in Table VI.†

The results show that the weight of precipitate varied from 0·8 milligramme to 4·3 milligrammes from each cubic centimetre.

Remarks on the Experimental Results.

When an amount of antiserum, *e.g.* 3 c.c., interacts in suitable dilution with increasing quantities of homologous protein, the weight of the precipitate augments as the quantity of homologous protein taking part in the interaction is increased (Table IV). The rate of increase in the weight of the precipitate is rapid as the quantity of homologous protein rises from minute quantities to 5 milligrammes or 10 milligrammes, the exact amount varying with different antisera. With further increments of homologous protein this rate of increase is diminished. When the quantity of homologous protein reaches from 30 milligrammes to 100 milligrammes (the exact amount varying with different antisera) any increase in the weight of the precipitate ceases.

* 'Journ. of Hygiene,' vol. 6, p. 262, 1906.

† An analysis of the effect of the various factors of the process of immunisation on the weight of the precipitate will be published later.

Table VI.

No. of antiserum.	Nature of protein used for immunisation.	Weight of precipitate from 1 c.c. antiserum.
		milligrammes.
42	Horse serum	1·4
43	"	
44	Ostrich egg-white	3·8
46	Fowl's egg-white	2·7
47	" "	
48	" "	
49	" "	2·4
50	" "	3·2
51	" "	3·9
52	" "	1·5
53	Horse serum	2·0
54	"	1·6
55	"	4·3
56	"	0·8
57	"	1·45
58	Fowl's egg-white	4·3
59	" "	4·3
60	" "	3·2
62	" "	2·2
63	" "	1·7
64	" "	1·5
65	" "	1·2
66	" "	1·16

Further increase in the quantity of the homologous protein (in these experiments up to 560 milligrammes protein) leads to no further increase in the weight of the precipitates (Tables I and II). With other amounts of antiserum, such as 1 c.c. or 5 c.c., interacting with increasing quantities of homologous protein, similar phenomena are observed. The weights of the precipitates rise to a maximum and then remain constant.

When the amounts of antiserum are varied, the weights of the precipitates are directly proportional to the amounts of antiserum as soon as the quantities of homologous protein are sufficient to produce the maximal precipitates (Table III). These quantities of homologous protein are those which neutralise completely the precipitin in the antiserum. The superfluids from such interactions fail to yield precipitates on the addition of any quantity of homologous protein. If the quantity of homologous protein be insufficient to neutralise all the precipitin in the antiserum, the weight of the precipitate is diminished and the addition of a further quantity of homologous protein to the superfluid leads to the formation of a precipitate. When the precipitin is neutralised in two or more stages by further increments of homologous protein, the weight of the combined precipitates is equal to that of the precipitate formed in a single interaction with a quantity of homologous protein sufficient to neutralise completely the precipitin (Tables II and III).

The quantities of homologous protein sufficient to neutralise the precipitin in varying amounts of the same antiserum show some relationship to the amount of antiserum. The quantities of homologous protein augment with an increase in the amounts of antiserum, but whether the quantities of homologous protein are directly proportional to the amounts of antiserum is not ascertainable from the present data. The weights of the precipitates formed when increasing amounts of antiserum interact with a constant quantity of homologous protein insufficient to neutralise the whole of the precipitin in the antiserum augment with the amounts of antiserum, but the weights of the precipitates from each unit of antiserum diminish as the amounts of antiserum increase (Table III, tubes Nos. 10, 11, 12, and 13).

The weights of the precipitates from 1 c.c. of different antisera are of value in showing the order of magnitude of the weights of precipitin taking part in precipitin interactions.

In conclusion, I beg to express my indebtedness to Prof. Anderson Stuart, in whose laboratory this research was carried out.

The Influence of Bacterial Endotoxins on Phagocytosis.

(Preliminary Report.)

By Leonard S. Dudgeon, P. N. Panton and H. A. F. Wilson.

(Communicated by Dr. F. W. Mott, F.R.S. Received April 26,—Read June 2, 1910.)

(From the Pathological Laboratories, St. Thomas's Hospital.)

These investigations were undertaken for the purpose of determining the effect of endotoxic substances on phagocytosis, as tested *in vitro.* Experiments were made to determine whether these substances, when added to a phagocytic mixture, would cause an increase or a decrease in the phagocytic activity; whether such action would be general or specific; whether the action would be affected by subjecting the endotoxins to varying degrees of temperature, and whether the toxins would act directly on the bacteria, the serum, or the leucocytes.

The present communication is intended only for the purpose of introducing our preliminary results, which are derived from a considerable amount of experimental enquiry. The explanation of the mode by which the effects to be described are produced is now under investigation.